RT Labeling with modified primers-10/1/02

I. RT Reaction

Primer (2 ug/ul)	2 ul
Total RNA (1-10 ug)	15.5 ul
RNasin (RNase inhibitor)	1 ul
Total	**18.5 ul**

Incubate RNA and primers at 70° C for 10 min. Chill on ice for 10 min
Bring to bench and incubate at RT for 10 min

cDNA synthesis: Using 5-(3-Aminoallyl)-2'-deoxyuridine 5'-triphosphate (SIGMA A-0410)

Component	ul	4 rnxs	8 rnxs	6 rnxs	12 rnxs
5X buffer	6	25.2	50.4	37.8	75.6
50X aa dUTP/dNTPs	0.6	2.52	5.04	3.78	7.56
DTT (0.1 M)	3	12.6	25.2	18.9	37.8
SSII RT	1.9	8	16	12	24
Total	**11.5**	**11.5x4.2**	**11.5x8.4**	**11.5x6.3**	**11.5x12.6**

50X aa dUTP/dNTPs: 10 ul each of dATP (100 mM), dGTP (100mM), and dCTP (100mM); 4 ul of aa dUTP (100 mM), 6 ul of dTTP (100mM)

Add the enzyme mix to above tube and incubate at 42 ° C for 2 hours

II. Hydrolysis

Add:	0 .5M EDTA	10 ul
	1N NaOH	10 ul

Incubate at 65°C for 30 min
Neutralize: 1M HCl 10 ul

III. Cleanup (with Qiagen's MinElute PCR Purification Kit)

Combine 300 ul of Buffer PB with 60 ul neutralized sample
Load the sample into MinElute column
Spin at 13K for 1 minutes. Reload the flo-thru and spin
Discard the flo-thru. Wash twice with 500 ul of PE buffer
Centrifuge at 13K for an extra minute to remove residual
Elute with 10 ul pHed H2O (1.5 ml H2O plus 5 ul I M NaHCO3, pH 9.3)
Incubate 1 min and spin at 13K for 1 min

Repeat the elution two more times

IV. Coupling

Dry down the above elute to 9 ul
Add 1ul of 1M NaBicarbonate Buffer pH 9.0 into elute
Add 4.5 ul NHS-cye dye resuspended in DMSO
Incubate at RT for 1 hour in dark

V. Quenching and Cleanup

Add 4.5ul 4M hydroxylamine
Incubate at RT for 30 min in dark

To remove unicorporated/quenched cye-dyes proceed with Qia-quick PCR purification kit

Combine Cy3 and Cy5 reactions
Add 60ul distilled water
Add 500ul Buffer PB
Apply to Qia-quick column and spin at 10K rpm for 1 min
Reload the column and spin for 1 min
Aspirate off flo-thru
Add 500ul Buffer PE and spin 30-60 sec
Aspirate off flo-thru and repeat
Aspirate flo-thru and spin for 1 min at high speed to dry column
Transfer to fresh eppendorf tube
Add 20ul Buffer EB and wait for 1 min at RT
Spin at 13,000 rpm for 1 min
Repeat elution step two more times

VI. Hybridization

Dry down Qia-quick eluate in speed vacuum.
Bring volume to 21 ul with water.

Add: 4.5 ul 20X SSC
2 ul of polyA (8 mg/ml)
1 ul of Cot-1 DNA (10 mg/ml)
1 ul of yeast tRNA (4 mg/ml)

Add: 0.5 ul 10% SDS.
Incubate reaction at 100° C for 2 min.
Spin at 13,000 rpm for 5 min at RT.
Apply to prepared microarray.